Treatment of Acute Respiratory Distress Syndrome with Recombinant Surfactant Protein C Surfactant

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We performed a phase I/II trial in North America of a recombinant surfactant protein C–based surfactant (Venticute) as treatment for the acute respiratory distress syndrome. Patients were prospectively randomized to receive either standard therapy or standard therapy plus one of two doses of exogenous surfactant given four times over 24 hours. Surfactant administration was well tolerated. No significant treatment benefit was associated with surfactant treatment. Bronchoalveolar lavage of treated patients at 48 hours reflected the presence of exogenous surfactant components, did not show evidence of improved surface tension lowering function, and had interleukin-6 concentrations that were significantly lower than control group values, consistent with an antiinflammatory treatment effect. The presence of exogenous surfactant was not detected in lavage fluid obtained at 120 hours. Future studies might rationally employ larger surfactant doses and a more prolonged dosing schedule.

Keywords: pulmonary surfactant; surfactant apoprotein C; phospholipids; interleukin-6

Acute respiratory distress syndrome (ARDS) is an acute inflammatory pulmonary injury in which marked edema of the lungs results in progressive impairment of gas exchange, atelectasis, and decreased lung compliance. Function of the lung surfactant system is impaired in patients with ARDS, and this impairment may contribute to atelectasis and decreased pulmonary compliance (1–4). The loss of surfactant function is multifaceted and causes include injury to the type II cells of the alveolar epithelium that produce surfactant components, inhibition caused by proteins in alveolar edema fluid, conversion of functional surfactant forms in the alveolar space to relatively inactive forms, and alteration of surfactant components by inflammatory processes (5).

Rationale for the use of exogenous surfactant in the treatment of patients with ARDS rests on multiple observations (6). First, the function of surfactant recovered in bronchoalveolar lavage fluid (BAL) from patients with ARDS and from animal models of acute lung injury is greatly impaired. Second, administration of surfactant to animal models of ARDS results in improved gas exchange. Third, treatment of infants with respiratory distress syndrome and impaired surfactant function is associated with improved survival. This improvement is observed even in the case of established respiratory distress syndrome in which significant lung inflammation is present (7, 8). Fourth, surfactant modifies the production by neutrophils of reactive oxygen species (9) and also modulates immune responses (10). These actions may be of benefit in the setting of intense pulmonary inflammation that is characteristic of patients with ARDS.

Finally, several clinical trials have suggested that treatment of ARDS patients with exogenous surfactant may provide benefit (11–16). In these trials, surfactant from natural sources (containing surfactant proteins) was administered by instillation. In contrast, a synthetic protein-free surfactant administered in very low doses by aerosolization was studied in a large multicenter randomized trial, and no effect on survival at 30 days was demonstrated (17).

Because inclusion of hydrophobic surfactant proteins may be critical for optimal function and because instillation is the only mechanism currently available for delivering adequate amounts of surfactant, we studied the effects of a recombinant surfactant protein C (rSP-C, lusupultide)–based surfactant (Venticute; ALTANA Pharma AG, Konstanz, Germany) in an animal model of ARDS before this clinical study. We determined dosage, instillation techniques, and volumes that optimally improve gas exchange, and these preclinical observations were then used to design the phase I/II study reported here (18, 19). In addition, we chose to deliver four instillations, as prior experience with a natural surfactant did not support the use of additional doses (13). The study reported here had two objectives: to assess the safety and efficacy of two dose levels of rSP-C surfactant and to assess BAL recovered from patients for the presence and function of surfactant components and for evidence of treatment effect on pulmonary inflammation.

METHODS

Complete details of study methods are provided in the online supplement. The study was approved by local institutional review boards at each participating institution.

Study Design

Patients were 18 or more years old, had ARDS as defined by the American European Consensus Conference (20) for not more than 48 hours, required at least 5-cm positive end expiratory pressure, and had associated burn injury, trauma or surgery, polytransfusion, aspiration, sepsis syndrome, pancreatitis, inhalation injury, or pneumonia.

During a 6-hour baseline period, clinical and respiratory parameters were recorded, and BAL was obtained. Volume-controlled ventilation using tidal volumes of 6–10 ml/kg was employed throughout the study.

Surfactant Administration

Patients received a rSP-C (lusupultide)–based surfactant (Venticute) that contains (wt/wt) 1.8% rSP-C, 63.4% 2-dipalmitoyl-sn-s-phosphatidylcholine, 27.8% 1-palmitoyl-2-oleoyl-3-sn-phosphatidylglycerol, 4.5% palmitic
acid, and 2.5% CaCl2. Patients were randomized to receive 1 ml of rSP-C surfactant (containing 1 mg of rSP-C + 50 mg of phospholipid) per kilogram lean body weight up to four times in 24 hours (group HIGH, n = 15), 0.5 ml of rSP-C surfactant per kilogram lean body weight up to four times in 24 hours (group LOW, n = 12), or no drug (group CON, n = 12). Surfactant was administered through a catheter inserted through the endotracheal tube and was advanced to within 1–2 cm of the carina. Patients were placed in the right lateral decubitus position with the head elevated 30°, and aliquots of up to 25 ml were delivered over 20–30 seconds with the ventilator paused at end exhalation. After half the dose had been administered, the patient was placed on the left side, and the process was repeated until the entire dose was administered. Up to three additional doses were administered at predefined time points during the 24 hours after initial treatment.

**Follow-up Observations and Weaning**

Patients were observed for up to 28 days or until hospital discharge. Patients were screened daily using published guidelines for ability to wean from the mechanical ventilator (21).

**Analysis of Clinical Samples**

Blood was collected for analysis, and BAL fluid was obtained before treatment and 48 and 120 hours after treatment for analysis of surfactant components and function and for measurement of inflammatory mediators. Experiments were performed to assure that components of BAL obtained after treatment did not affect the detection of interleukin (IL)-6.

**Study Endpoints and Statistical Analysis**

Two primary variables were defined prospectively. The first, a measure of gas exchange, was the excess area under the PaO2/FiO2-versus-time curve for the 24-hour period beginning with administration of the first surfactant aliquot (for patients in the HIGH and LOW groups) or for the 24-hour period beginning 1 hour after randomization (for the CON group). The area under the curve from 0 to 24 hours was the area between the horizontal line corresponding to the average of the baseline PaO2/FiO2 values and the linearly connected PaO2/FiO2 values measured at time points during the 24 hours after t = 0. The second primary variable, a measure of clinical effect, was the number of days of unassisted breathing during the 28-day observation period (ventilator-free days). Intention-to-treat analysis was performed for each of these variables.

Differences among normally or nonnormally distributed group data were detected using the analysis of variance or Kruskal-Wallis statistic, respectively; differences between treated and control groups were detected using the Mann-Whitney U test (22). Values are presented as mean ± SEM or median and 25–75-per centile range unless otherwise noted.

**RESULTS**

Forty patients were enrolled. The number of patients enrolled per study site varied from one (four sites) to eight (one site). Baseline data for each of the study groups are shown in Tables 1 and 2. No significant differences among groups were detected. Values for the modified Acute Physiology and Chronic Health Evaluation II score indicate a similar severity of illness among study groups. Because evaluation of neurologic function and chronic health status is omitted from this modified score, values cannot be compared directly to values from studies using the unmodified Acute Physiology and Chronic Health Evaluation II score. In the HIGH and LOW groups, all four doses of surfactant were administered to 93.3% and 100% of the patients, respectively.

**Clinical Results**

**Safety.** There were no significant differences among study groups in the frequency of all adverse events or of serious adverse events. Only one adverse event, transient hypoxia and supraventricular tachycardia, was judged likely to be related to treatment. No adverse event caused administration of rSP-C surfactant to be curtailed.

**Gas exchange.** Values for the excess area under the PaO2/FiO2 curve during the first 120 hours after treatment are presented in Table 3. No significant difference among groups was apparent at 24 or 120 hours.

**PaO2/FiO2** values for the three groups are shown in Figure 1. Differences between either treated group and the CON group were not significant. As ventilation strategies may influence gas exchange, mean tidal volumes per kilogram body weight and end-inspiratory plateau pressures during the first 120 hours after treatment were examined; no significant differences among groups were detected (Table E1, online supplement).

**Outcomes.** The median number of ventilator-free days for each of the three study groups is shown in Table 3. Differences between either treated group and the CON group were not significant. The number of patients weaned, surviving at Day 28, and alive on the ventilator at Day 28 is also shown in Table 3.

**BAL Analyses**

Surfactant concentration, composition, and function. Median BAL phospholipid concentrations increased in the two treated groups by approximately 50% at 48 hours, although these differences did

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON</th>
<th>HIGH</th>
<th>LOW</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Age, yr*</td>
<td>51 ± 5</td>
<td>59 ± 5</td>
<td>52 ± 5</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>5/8</td>
<td>8/7</td>
<td>4/8</td>
</tr>
<tr>
<td>Modified APACHE II*</td>
<td>10.9 ± 1.1</td>
<td>10.2 ± 1.2</td>
<td>10.1 ± 1.7</td>
</tr>
<tr>
<td>Predisposing events, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burn</td>
<td>1 (8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Trauma/surgery</td>
<td>1 (8)</td>
<td>4 (27)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Polytansfusion</td>
<td>0 (0)</td>
<td>1 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Aspiration</td>
<td>2 (16)</td>
<td>4 (27)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Sepsis syndrome</td>
<td>7 (54)</td>
<td>4 (27)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Pancrtitis</td>
<td>0 (0)</td>
<td>1 (7)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>6 (46)</td>
<td>5 (33)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>Hours from diagnosis to initial treatment*</td>
<td>23.6 ± 3.7</td>
<td>36.5 ± 8.2</td>
<td>28.8 ± 3.6</td>
</tr>
</tbody>
</table>

**Definition of abbreviation: APACHE = Acute Physiology and Chronic Health Evaluation.**

* Mean ± SEM.
not reach statistical significance because of substantial within-group variability (Table E2, online supplement). Fractional content of phosphatidylcholine in BAL from the HIGH group was significantly greater than from the CON group at 48 hours (68.4% versus 50.4%). As rSP-C surfactant has a fractional content of phosphatidylglycerol that is much greater than that of native surfactant, changes in the fractional content of phosphatidylglycerol in surfactant recovered in BAL are likely to reflect the presence of rSP-C surfactant. As shown in Figure 2A and Table E2, the fractional content of phosphatidylglycerol, as well as the absolute amount of phosphatidylglycerol, was increased significantly in both treated groups 48 hours after the initiation of treatment. This resulted in significantly lower phosphatidylcholine/phosphatidylglycerol ratios in BAL samples obtained from both surfactant treatment groups compared with the CON group at 48 hours (Table E2). Similarly, because the only surfactant protein in rSP-C surfactant is SP-C, increases in BAL SP-C content are also likely to reflect the presence of rSP-C surfactant. As shown in Figure 2B and Table E2, significant increases of SP-C were observed at 48 hours in BAL from the HIGH and LOW group patients relative to CON patients. No significant differences between control and treated groups in BAL concentrations of surfactant protein A or surfactant protein B or in the fractional content of large surfactant aggregates were detected (Table E2).

Surface tension lowering function of surfactant recovered from BAL, measured as surface tension after 15 seconds adsorption or as minimum surface tension after 10 minutes of bubble oscillation in the bubble surfactometer, was not significantly different between either treated group and control (Table E2). A sufficient quantity of surfactant was available for analysis in samples obtained at 0, 48, and 120 hours, respectively, from 9, 4, and 4 patients in the CON group; 10, 7, and 3 patients in the HIGH group; and 4, 6, and 1 patient in the LOW group.

**Protein and cell constituents.** Median total BAL protein concentrations ranged from 0.3 to 1.17 mg/ml and did not differ significantly between either treated group and CON at any time point (Table E3, online supplement).

IL-6 levels were markedly decreased at 48 hours in BAL from both treated groups compared with values from the CON group (Figure 3), and this decrease was significant for the HIGH group (Figure 3 and Table E2).

No differences between either treated group and CON in BAL cell differential or levels of α1-proteinase inhibitor, neutrophil elastase, myeloperoxidase, IL-1β, IL-8, or transforming growth factor-β1 were detected (Tables E3 and E4, online supplement). IL-10, IL-13, platelet-derived growth factor-β, and tumor necrosis factor-α were not detected in the majority of these unconcentrated BAL samples.

**Analyses of Blood and Blood Plasma**

Plasma IL-6 levels in the HIGH group were significantly decreased relative to the CON group when measured at 72 hours (Figure 3B).

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**TABLE 3. CLINICAL RESULTS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON</th>
<th>HIGH</th>
<th>LOW</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>AUC0–24 hr, mm Hg · hr</td>
<td>440 (97–1,304)</td>
<td>247 (–59–902)</td>
<td>446 (151–841)</td>
</tr>
<tr>
<td>AUC0–120 hr, mm Hg · hr</td>
<td>1698 (900–6,683)</td>
<td>2661 (2,592–7,457)</td>
<td>2231 (3–5,380)</td>
</tr>
<tr>
<td>VFD</td>
<td>6 (0–15)</td>
<td>5 (0–18)</td>
<td>4 (0–12)</td>
</tr>
<tr>
<td>Patients weaned and alive on Day 28, n (%)</td>
<td>7 (54)</td>
<td>7 (47)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Patients alive, Day 28, n (%)</td>
<td>8 (62)</td>
<td>12 (80)</td>
<td>8 (67)</td>
</tr>
</tbody>
</table>

**Definition of abbreviations:** AUC0–24 hr = area under the curve from 0 to 24 hours; AUC0–120 hr = area under the curve from 0 to 120 hours; VFD = ventilator-free days to Day 28.

Data are presented as median (25–75 percentile) unless otherwise noted.

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**Figure 1.** The PaO2/FIO2 values for the CON (diamonds), HIGH (squares), and LOW (triangles) groups are shown for each study time-point. Data are mean ± SEM.

**Figure 2.** Median values for the fractional content of phosphatidylglycerol (PG) in BAL phospholipids (PL) (A) and median concentrations of SP-C in BAL (B) are shown for the CON (dark gray bars), HIGH (open bars), and LOW (light gray bars) groups. The presence of a significantly increased fractional content of PG and of significantly increased levels of SP-C at 48 hours in the treated groups is consistent with the recovery of rSP-C surfactant components in BAL. Error bars denote the 25th and 75th percentiles. Values differing significantly from CON (p < 0.05) are indicated by an asterisk.
DISCUSSION

This phase I/II trial was conducted to discover if an rSP-C–based surfactant could be administered safely to patients with ARDS and whether such administration might provide indication of therapeutic efficacy. Study of an rSP-C–based surfactant is of interest, as its use in lung injury models has been associated with improvement in gas exchange that may exceed that associated with natural surfactants (18, 19, 23). In addition, SP-C levels have recently been shown to be significantly depressed in BAL recovered from patients with ARDS (24). Finally, in contrast to natural surfactants, synthetic surfactants have defined composition and avoid the risk of transmitting infectious agents.

The adverse events reported during this study are those expected in patients with ARDS and did not prevent administration of the study drug. In particular, high ventilating pressures and significant decrements in blood oxygenation were not observed.

Statistically significant evidence of clinical benefit was not observed in this pilot study. The HIGH group, compared with the CON group, had greater survival at 28 days and greater area under the PaO2/FiO2-versus-time curve to 120 hours, providing rationale for future study of surfactant treatment of ARDS patients. The optimal strategy for administering exogenous surfactant to patients with acute lung injury (ALI) is poorly defined, and this study also provides information that may help in developing future strategies. Surfactant biophysical function (as reflected in surface tension-lowering ability of surfactant recovered from BAL 48 or more hours after the final treatment) was not significantly improved, and thus, larger doses of rSP-C surfactant may be required to overcome inhibition of biophysical function by proteins in airway fluid. Although the concentration of phospholipid in BAL may reflect variables other than the amount of surfactant present in the lower airway, studies in which larger doses of exogenous surfactant have been administered have shown significant increases in BAL phospholipid concentration measured at 120 hours (13). Thus, the lack of significant increase in BAL phospholipid concentrations that we observed in treated patients at both 48 and 120 hours also suggests the need for administration of larger amounts of exogenous surfactant.

The frequency of surfactant administration is also likely to be an important variable. The increased fractional content of phosphatidylglycerol and increased levels of SP-C detectable in BAL obtained 48 hours but not 120 hours after the initial administration suggests that exogenous rSP-C surfactant was not adequately retained in the lung. Thus, it may be beneficial in future studies to administer surfactant over periods longer than 24 hours—perhaps over periods of 120 or more hours.

Exogenous surfactant might provide therapeutic benefit in several ways. First, recruitment of nonventilated alveoli may improve gas exchange and allow use of nontoxic concentrations of oxygen. Second, reduction of surface tension may decrease local mechanical forces. Recent observations have established the value of ventilation strategies that minimize lung stretch and airway pressure (25), and thus, surfactant treatment may also have value through reduction of these mechanical forces. Finally, there is an increasing body of evidence that lung surfactant may modulate innate immune mechanisms. This modulation is likely to occur through interaction of surfactant protein A and surfactant protein D with cellular and bacterial elements (26). However, rSP-C surfactant contains neither surfactant protein A nor surfactant protein D and thus would not be expected to affect this interaction. However, surfactant lipids have also been shown to alter inflammation through modulation of phagocyte function (9), and thus, rSP-C surfactant may affect local inflammation through this mechanism.

Levels of IL-6 in BAL or plasma of patients with ALI have been interpreted as reflecting the inflammatory state of the lung.
and have been correlated with survival in studies of ARDS patients (27). Patients receiving low-stretch ventilation have been shown to have a significant fall in these levels compared with changes observed in patients receiving conventional ventilation (25, 28). We found that the IL-6 levels in BAL acquired 48 hours after study initiation were significantly lower in surfactant-treated than in control patients. This change might occur through either direct or indirect mechanisms. Surfactant containing only dipalmitoylphosphatidylcholine, cetyl alcohol, and tyloxapol, as well as surfactant containing dipalmitoylphosphatidylcholine, surfactant protein B, and SP-C, downregulated leukocyte production of IL-6 (29). Thus, it is likely that dipalmitoylphosphatidylcholine (the common component) might downregulate leukocyte production of not only reactive oxygen species but also of IL-6. The rSP-C surfactant delivered to patients in this study might have acted directly on leukocytes present in the lung to affect directly their production of IL-6. Alternatively, surfactant treatment could affect IL-6 levels indirectly by decreasing mechanical forces in the lung—with an associated decrement in inflammation that is reflected in lower IL-6 levels—much as reported previously in studies of low-stretch ventilation (25, 28).

In summary, this study provides evidence that rSP-C surfactant may be safely administered to patients with ALI. Data from this study have been used in the design of two phase III studies. Preliminary reports from these studies show that in patients with ARDS caused by a variety of predisposing events, treatment with rSP-C surfactant resulted in improvement in blood oxygenation (30). Outcome at 28 days was not affected. Taken together, this experience suggests that future studies might rationally be designed to employ higher doses of surfactant and/or a longer treatment period.

Acknowledgment: The authors recognize the outstanding analytic efforts of Ms. Kathryn Harris.

References